Journal of Soil Science and Plant Nutrition, 2016, 16 (2), 438-460
RESEARCH ARTICLE

Phosphorus response and optimum pH ranges of twelve pasture legumes grown in an acid upland New Zealand soil under glasshouse conditions

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Abstract

Pasture legumes provide critical nitrogen inputs but fail to persist in typical acidic low P fertility upland soils. Alternative legume species for these environments are urgently sought. Twelve novel legume species were grown for 42 weeks under glasshouse conditions in an acidic upland New Zealand soil. Phosphorus (P) was applied at eight rates (0, 10, 30, 60, 100, 250, 500, 1500 mg kg^{-1} soil) or lime (100% $CaCO_3$) at five rates (0, 2, 5, 8, 15 t ha^{-1} equivalent). Annual species grew on average for 25 weeks, while perennial species grew for 42 weeks. Yield was measured and herbage was analysed for macro and micro elements and soils analysed for pH, exchangeable Al and Olsen P. P responses differed substantially between legume species which were likely driven by genetic adaptations to low phosphorus environments. Critical shoot P concentrations for optimum yield were identified and ranged from 0.23 (tagasaste) to 0.39 % P (falcata lucerne). Arrow leaf, subterranean and balansa clovers had the greatest yield increase at low P inputs (100 mg P kg^{-1}) and show promise as alternatives to white clover. Lime treatments increased the yield of all species to a point beyond which yield decreased with further additions. Increased yield was primarily driven by decreased soil exchangeable aluminium (Al) concentrations and to a lesser extent by increased soil P and molybdenum (Mo) availability, while decreases were driven by lower soil P and boron (B) availability at high pH. Thresholds for soil exchangeable Al differed between legume species. Species with higher Al thresholds (7 – 8 mg Al kg^{-1}) were tagasaste, lotus, persian and gland clovers and falcata lucerne. These species show promise for acid soil environments with soil Al issues and should now be investigated further in field experiments. For many of these legume species this represents new and critical information.

Keywords: Phosphorus, pH, soil exchangeable Al, legumes, pasture, yield response

1. Introduction

Pasture legumes play critical dual roles in grazed grassland ecosystems; critical nitrogen (N) inputs via fixation of atmospheric N_2 (Goh and Bruce, 2005) and the production of high quality feed for grazing rumenants (Haynes and Williams 1993). Traditionally, white clover (*Trifolium repens*) has been the legume of choice in legume / grass pastures. However in dryland agriculture,white clover fails to persist resulting in low yielding, N starved grassland with low stock carrying capacity (Scott 2003). Dryland swards of low clover content are now common place in New Zealand (NZ), severely restricting productivity (Brown and Green 2003). Key factors driving the presence of legumes in the field include soil fertility and moisture availability (Moir and Moot 2010, 2014). Soil phosphorus (P) and pH are often low in dryland, both of which have negative impacts on pasture legumes (Maxwell *et al.* 2013). However, very little information exists in literature on P efficient and pH tolerant legumes which may provide alternatives to white clover.

The upland (generally land above 300 masl) of N.Z are extensively farmed and often have a short, moisture limited production season. The soils are typically acidic, with low available phosphorus (P) and sulphur (S) which influences the growth and persistence of legumes (Haynes and Williams 1993; Moir *et al.*1997, 2000). Legumes play a key role in New Zealand agriculture, contributing the key source nitrogen (N) to the system as well as increasing the quality of feed, for improved animal performance (Brown and Green 2003). There is extensive literature on the optimum growth conditions of white clover (*Trifolium repens*) (Black *et al.* 2000; During and Brier 1973; Lowther and Adams 1970), the most commonly sown clover in New Zealand. As such white clover is poorly suited to dryland low fertility conditions and performs well in high fertility soils at pH 6.0 or higher in high rainfall environments (Black *et al.* 2000). Subterranean clover (*Trifolium subterranean*) has also been researched for this environment (Brown *et al.* 2006; Hayes *et al.* 2008a). However, farmers require a more diverse range of both perennial and annual legume species for their dryland farming systems (Brown and Green 2003). There is little available information on many other potential species for upland environments in terms of optimum soil or nutrient conditions for growth.

Phosphorus is required at higher concentrations for legumes to grow and compete with grasses (Caradus 1980). Adequate plant-available soil P is critical for legume production and persistence. P has been aerially applied to upland in the form of superphosphate for over 50 years (Gillingham *et al.* 1999). However, due to the economics of this practice, most upland soils are deficient in P (Nguyen and Goh 1992). For this reason species which perform well in low P soils are valuable for this type of production system and environment. However, literature reporting optimum soil P concentrations (requirements) for alternative pasture legume species is scarce.

Pasture legumes are also sensitive to acidic (low pH) soil conditions (Edmeades and Ridley 2003) particularly in terms of aluminum (Al) toxicity which severly affects plant establishment, yield and persistence. As such, soil acidity and related aluminium (Al) toxicity represent serious impediments to legume abundence and survival in grazed grasslands (Moir and Moot 2014). The application of lime is used to increase soil pH in agricultural systems. However this practice is often uneconomic on extensive farms due to high aerial application costs (Maxwell *et al.* 2014). Legumes which are able to persist and thrive in low pH soils, or with low inputs of lime and P, would be well suited to this environment and could be of major benefit to dryland farming systems. However, similar to plant P information, literature reporting soil pH tolerance and optimum soil pH ranges for individual legume species is scarce.

The objective of this study was to determine the effects of P or lime addition on the growth and nutrient uptake of twelve legume species grown in an acid upland soil under glasshouse conditions. The hypothesis being tested is that the growth and nutrient uptake will differ between species underdefined soil P and pH regimes.

2. Materials and Methods

2.1. Soil collection and preparation

Soil (0–0.15 m depth) was collected from an upland site (48° 08' 25" South. 172° 11' 20" East; 430 m.a.s.l.; 600 mm annual rainfall) on 'Mt Pember Station' in the Lees Valley, North Canterbury, New Zealand in May 2010. The soil is a upland brown shallow stony soil (NZ classification: Orthic brown soil, (Hewitt 1998); USDA: Dystrochrept, (Soil Survey Staff 1998)). This site has not received fertilizer or lime since the start of livestock grazing on the property over 100 years ago. The pasture is dominant in low fertility species such as browntop (*Agrostis tenuis*) and has very low (< 1%) legume content. The soil was prepared by passing it through a 4 mm sieve while field moist, removing all plant material, and then mixing thoroughly. Subsampleswere prepared for analysis by air-drying (30 °C for 7 days, then 2 mm sieved).

2.2. Soil chemical analysis

Soil fertility analyses were conducted and the results presented in Table 1, with cited methodologies.

2.3. Experimental design and trial management

A pot trial was conducted under glasshouse conditions using a complete randomized block design, examining 12 species of pastoral legumes. Eight rates of phosphorus and five rates of lime were used: 0, 10, 30, 60, 100, 250, 500 and 1500 mg P L^{-1} (Olsen P 7, 10, 12, 14, 18, 32, 60 and 181 mg P L^{-1} respectively), or 0, 2, 5, 8 or 15 T lime ha^{-1} (pH_{H_2O} 5.0, 5.3, 6.0, 7.0 and 7.5 respectively). When soil Olsen P and pH were measured at the end of the experiment, values had not changed since the commencement. Phosphorus treated pots received basal lime (5 T ha^{-1}, pH 6.0), while limed pots received basal P (250 mg P L^{-1}, Olsen P 30 mg P L^{-1}). Fertiliser treatments were mixed with 600 g of fresh field-moist soil and added to pots 746 cm^3 in volume in June 2010, then wetted up. The mean daily temperature for the trial period was 19.4 °C with daily average temperatures ranging between 16.2 and 23.9 °C. The 12 legumes investigated included five annual and seven perennial species (Table 2). Seeds (10 pot^{-1}) were sown in June thinned to the final plant density of 5 plants pot^{-1} in mid-July. The pots were inoculated with commercial (diluted peat culture) rhizobia strains (Table 2) 16 days post germination to insure that an active soil rhizobia population was present. Basal nutrient solution was applied to the pots throughout the experiment. The nutrient solution (Booking 1976; Caradus and Snaydon 1986) which was applied to the lime treatment pots was a 'semi-complete solution', containing all macro and trace elements required for plant growth. The P treatment pots received a modified version of this solution, which contained no P. These solutions were applied on a regular basis (twice monthly over winter, then weekly thereafter during rapid growth in spring and early summer) to ensure adequate plant nutrition.

In terms of key macro nutrients, the pots received a total of 200 kg S ha^{-1} and 500 kg K ha^{-1} from the nutrient solutions. The lime pots received approximately 200 kg P ha^{-1}, which increased the soil Olsen P to an optimum level of 30 mg P L^{-1}. Plants were dependant on N sourced from N fixation or soil N for growth. The pots were watered daily to 35-40% volumetric water capacity which represented a soil which was neither waterlogged nor dry.

Table 1. Initial soil test results for the upland brown shallow stony soil

Soil Analysis	Value	By method of
pH	5.1	Blackmore *et al.* (1987)
Olsen P	7 mg L^{-1}	Olsen *et al.* (1954)
Sulphate Sulphur	9 mg kg^{-1}	Searle (1979)
Ext. Org. Sulphur	8 mg kg^{-1}	Blackmore *et al.* (1987)
Reserve Potassium	6.0 me $100g^{-1}$	Metson *et al.* (1968; 1956)
P Retention	55%	Blakemore *et al.*(1972)
Anaerobic MinN	125 kg ha^{-1}	Keeney and Bremner (1966).
Exchangeable Aluminium	14 mg kg^{-1}	Edmeades *et al.* (1983)
Reserve Magnesium	23.6 me $100g^{-1}$	Metson (1975)
Total Nitrogen	0.41% w/w	
Total Carbon	5.35% w/w	Horneck and Miller (1998)
Carbon/Nitrogen	13:1	
Total Phosphorus	904 mg kg^{-1}	Blackmore *et al.* (1987)
CEC	15 me $100g^{-1}$	Hesse (1971)
Calcium	1.40 me $100g^{-1}$	
Magnesium	0.49 me $100g^{-1}$	Schollenberger and Simon (1945)
Potassium	0.41 me $100g^{-1}$	
Sodium	0.14 me $100g^{-1}$	
Base Saturation (Total)	16.1%	Hesse (1971)

Table 2. Legume species, seed source and rhizobium inoculation group used

Legume Species	Common Name and Cultivar	Source	Rhizobium Group*
1. Annual Species			
Trifolium vesiculosum	Arrowleaf clover cv. 'Cefalu'	West Coast Seed Ltd (Aus)	C
Trifolium michelianum	Balansa clover cv. 'Bolta'	Seed Mark Ltd	C
Trifolium glanduliferum	Gland clover cv. 'Prima'	Kiwi Seed Co Ltd	C
Trifolium resupinatum	Persian clover cv. 'Enrich'	Specialty Seeds Ltd	C
Trifolium subterraneum	Subterranean clover cv. 'Mt Barker'	Kiwi Seed Co Ltd	C
2. Perennial Species			
Trifolium aumbiguum	Caucasian clover cv. 'Endura 3'	PGG Wrightsons Ltd	CC238b
Lotus pedunculatus	Lotus cv. 'Grasslands Maku'	Specialty Seeds Ltd	D
Medicago falcate	Falcata lucerne	Kiwi Seed Co Ltd	AL
Medicago sativa;	Lucerne cv. 'Force 4'	Seed Force Ltd	AL
Trifolium fragiferum	Strawberry clover cv. 'Lucila'	Gentos ARG	B
Chamaecytisus proleferus	Tagasaste	Collected near Lincoln, New Zealand	B/C/D/AL
Trifolium repens	White clover cv. 'Grasslands Nomad'	PGG Wrightsons Ltd	B

*All rhizobium supplied by Becker Underwood Ltd, Australia (,Nodulaid,) *2.4. Measurements*

Shoots were harvested every four to five weeks, from September to November 2010 (annual species), or to April 2011 (perennial species) so as to maximise shoot yield, but prevent plants from flowering (becoming reproductive). Once harvested all herbage samples were oven dried at 70 °C for 48 hours, weighed, finely ground, bulked on a pot basis, then underwent acid digestion and nutrient analysis herbage (0.1000 g) was digested in 2 ml of nitric acid (HNO_3) on a heating block at 110 °C for two hours then 5 ml deionised water added before analysis. Digest samples were analysed for a complete range of elements (excluding N) using ICP-OES analysis (Varian 720-ES ICP-OES; Varian Inc., Victoria, Australia). All soil from pots was dried (30 °C, 7 days) for final soil analyses, sieved (2 mm) and a range of soil analyses conducted.

2.5. Statistical analysis

All data were tested for treatment effects by conducting an analysis of variance (ANOVA) using Genstat version 13.0 (VSN International). For the P treatment pots, the model included plant species, P rate and species × P rate interaction as fixed effects, with shoot yield, shoot P concentration and P uptake as random effects. For the lime treatment pots, the model included plant species, lime rate and species × lime rate interaction as fixed effects, with shoot yield, shoot P concentration and uptake and shoot Mo and B concentration as random effects. In some instances a statistically significant species × (P rate / lime rate) interaction was detected and regression analysis (curve fitting) was used to understand these relationships, where appropriate, using Sigmaplot 11.0 (Systat Software Inc. GmbH. 2008).

In general, fitted response curves were 'Mitsherlich' type in form; while some of the lime response curves were bell-shaped (full details are presented in the supplementary material).

3. Results

3.1. Phosphorus applications

3.1.1 Olsen P

The relationship between P rate and soil Olsen P is presented in Figure 1. There was an increase in Olsen P from 7 µg ml^{-1} at 0 mg P kg^{-1}, to 181 µg ml^{-1} at 1500 mg P kg^{-1}. This was an increase in Olsen P of 0.12 µg P ml^{-1} per mg P applied kg^{-1} soil. Between 0 mg P kg^{-1} and 10 mg P kg^{-1} the Olsen P increased 0.20 µg P ml^{-1} per mg P applied kg^{-1} soil. While between 500 mg P kg^{-1} and 1000 mg P kg^{-1} the Olsen P increased by 0.12 µg P ml^{-1} per mg P applied kg^{-1} soil.

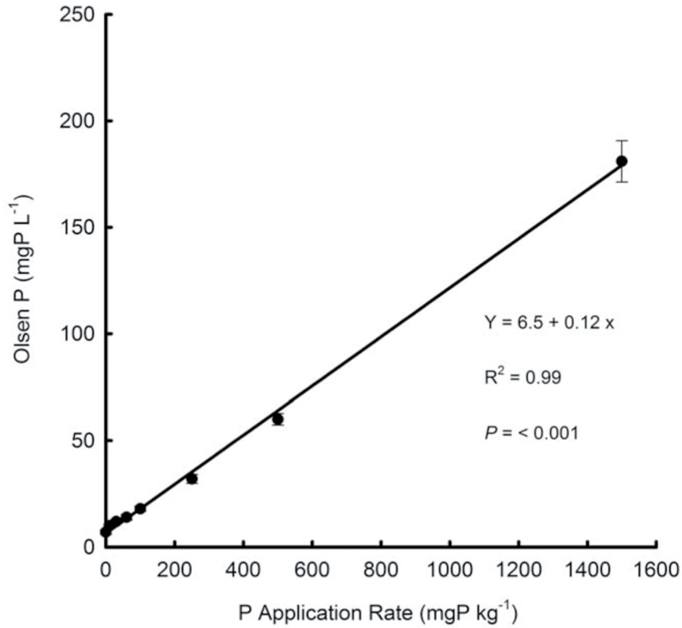
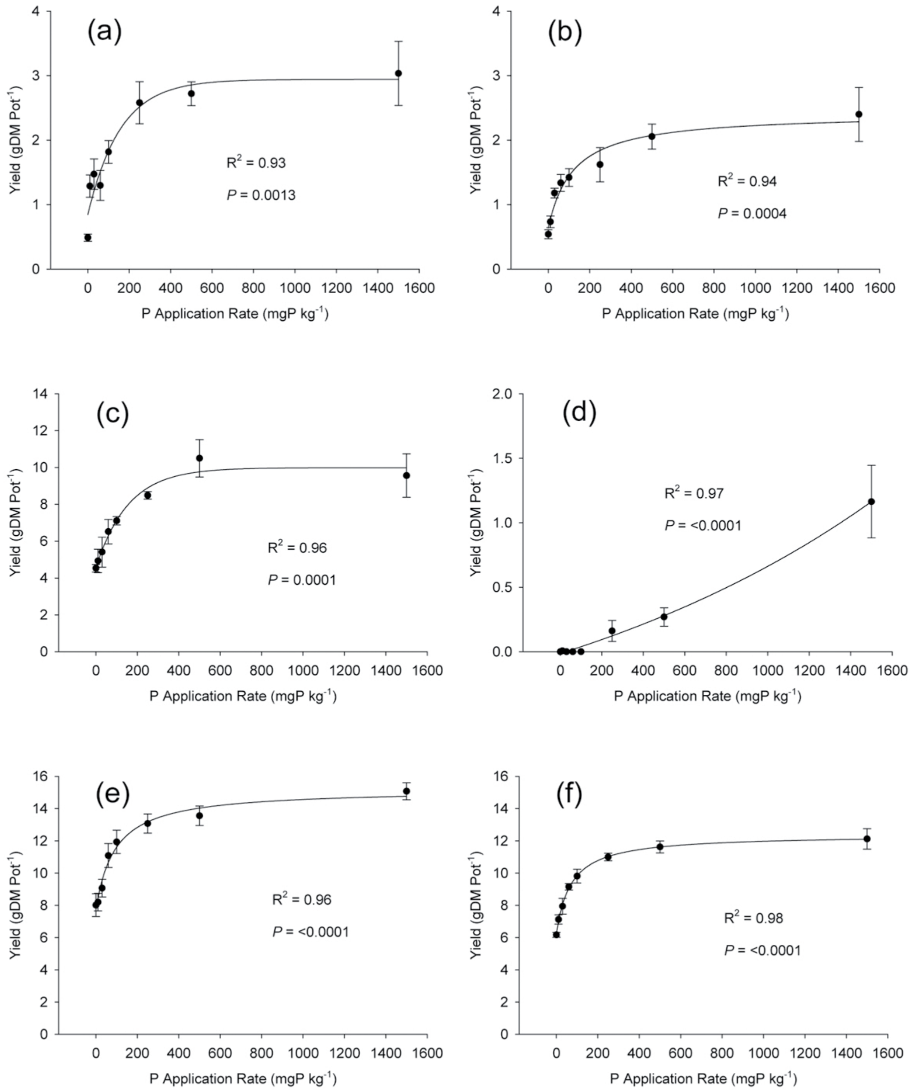


Figure 1. The relationship between P application rate (8 levels of P; ranging from 0 to 1500 mg P kg^{-1} soil) and soil Olsen P. Soil pH = 6.0. Data are mean values ± SEM (n=32), with P and R^2 values for fitted curves showing data trend.

3.1.2. Yield

A strong species difference (Table 3; $P< 0.001$) in dry matter (DM) yield was observed with increasing rates of phosphorus. Persian clover strongly out yielded other annuals (10 g DM pot^{-1}; Table 3). In terms of the perennial species, mean DM yields across all P treatments ranged from 6.1 g DM pot $^{-1}$ (white clover) to 11.3 g DM pot^{-1} (lotus). In most cases the perennials produced a greater total DM yield than the annual clovers. On average the greatest increase in DM yield occurred between 30 and 60 mg P kg^{-1} soil. There was a strong and highly significant ($P< 0.001$) species by P rate interaction. As such, these interactions represent the key results of the P treatments in this experiment. For this reason the relationship between increased P rate and DM yield was examined in more detail using regression analysis of the data for individual species. Most annual species followed a typical 'Mitscherlich' type yield response curve with increasing P rate (Figure 2). Yield was strongly associated (R^2 = 0.93 – 0.98) with P rate. The exception was gland clover, where the relationship between yield and P rate was best explained by an exponential curve function. The perennial species all followed a 'Mitscherlich' relationship with increasing P inputs, and again, these relationships were very strong (Figure 2). At 0 mg P kg^{-1} lotus, Persian clover and tagasaste were the highest yielding species at 8.0, 7.0 and 6.4 g DM pot^{-1}. Gland, arrow leaf, balansa and subterranean clovers were low yielding species at 0, 0.5, 0.5 and 0.9 g DM pot^{-1} respectively. At 1500 µg P pot^{-1} lotus, tagasaste and lucerne were the highest yielding species at 15.0, 13.0 and 12.1 g DM pot^{-1}. Gland, balansa and arrow leaf clovers were the lowest yielding at 1.2, 1.4 and 1.8 g DM pot^{-1}. At low P inputs (100 µg P kg^{-1}) arrow leaf clover, subterranean clover and balansa clover gave the greatest response in yield (above control).



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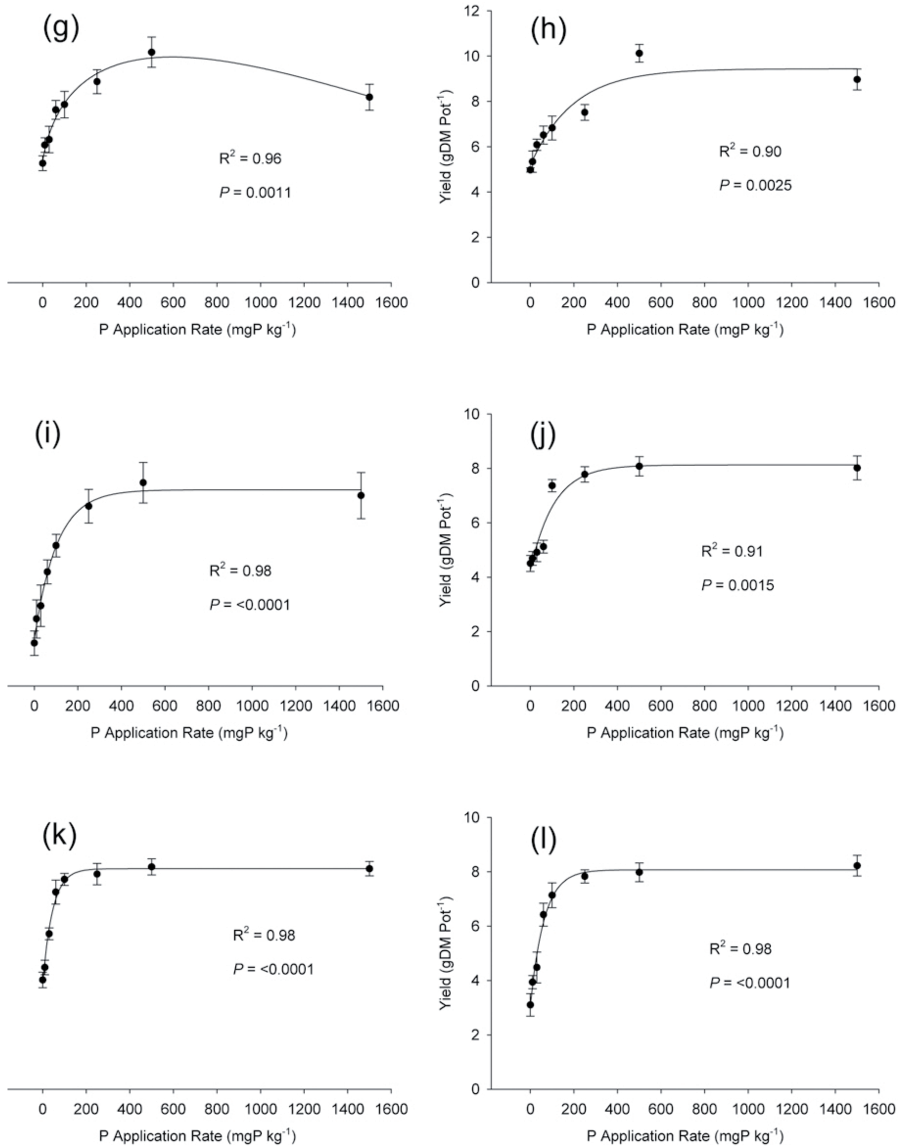


Figure 2 Total accumulated shoot dry matter (DM) yield response of pasture legume species (a) arrowleaf clover (*T. Vesiculosum*), (b) balansa clover (*T. michelianum*), (c) Caucasian clover (*T. ambiguum*), (d) gland clover (*T. glanduliferum*), (e) lotus (*L. pedunculatus*), (f) lucerne (*M. sativa*), (g) Persian clover (*T resupinatum*), (h) falcata lucerne (*M. falcata*), (i) subterranian clover (*T. subterranium*), (j) strawberry clover (*T. fragiferum*), (k) tagasaste (*C. proliferus*), (l) white clover (*T. Repens*) to increasing levels of soil phosphorus (8 levels of P; ranging from 0 to 1500 mg P kg^{-1}soil), grown in an acid NZ high country soil. Data are mean values ± SEM (*n*=4), with *P* and R^2 values for fitted curves showing data trend (Line equation for all graphs are presented in the supplementary material).

Table 3. Effects of P rates on mean shoot yields, P concentrations, P uptakes, Mo concentrations and B concentrations of five annual pasture legume species.

Species:		Shoot Yield (g DM Pot^{-1})	Shoot P (% P)	Shoot P Uptake (mg P Pot^{-1})	Shoot Mo (ppm)	Shoot B (ppm)
Arrow leaf clover		1.84	0.260	5.9	0..61	5.37
Balansa clover		1.41	0.261	4.2	0.84	8.45
Gland clover		0.20	0.079	0.6	0.54	3.33
Persian clover		10.06	0.210	22.2	0.63	15.82
Sub. Clover		2.81	0.249	7.9	0.60	8.66
Grand Mean		3.26	0.212	8.17	0.64	8.33
Species	SEM	0.131	0.0064	0.66	0.062	0.717
	LSD (5%)	0.368	0.0179	1.85	0.172	2.150
P Rate	0	1.78	0.125	2.5	0.52	6.87
	10	2.32	0.132	3.3	0.62	7.77
	30	2.57	0.144	4.0	0.43	6.85
	60	3.06	0.151	5.5	0.44	6.36
	100	3.36	0.168	6.6	0.54	6.13
	250	4.03	0.197	9.0	0.61	8.47
	500	4.62	0.257	13.0	0.99	9.21
	1500	4.34	0.522	21.7	0.99	14.94
	SEM	0.166	0.0081	0.66	0.078	0.907
	LSD (5%)	0.466	0.0226	1.85	0.218	2.720
P	Species	***	***	***	*	***
	P Rate	***	***	***	***	***
	Sp*P Rate	***	***	***	***	*

3.1.3. P uptake

The mean phosphorus concentration of shoots increased ($P< 0.001$; Tables 3 and 4) with increasing P inputs. Again, there was a strong and highly significant ($P< 0.001$) species by Prate interaction. Between species the mean shoot P concentration ranged from 0.079% (gland clover) to 0.288% (white clover). At 0 mg P kg^{-1} white clover and Caucasian clover had the highest P concentration at 0.220% and 0.227% while Persian clover had the lowest of the species that yielded, at 0.129%.

P uptake of the herbage across all species increased ($P< 0.001$) with increasing P inputs. There was an interaction between yield and herbage P concentration. The yield of the annual clovers increased with increasing P concentration in the shoot following a strong 'rise to maximum' relationship with the exception of Persian clover and gland clover (Table 3). Yield was strongly associated with P concentration. Lotus and white clover had the sharpest increase in yield with increasing herbage P concentration.

Table 4. Effects of P rates on mean shoot yields, P concentrations, P uptakes, Mo concentrations and B concentrations of seven perennial pasture legume species.

Species:		Shoot Yield (g DM Pot^{-1})	Shoot P (% P)	Shoot P Uptake (mg P Pot^{-1})	Shoot Mo (ppm)	Mean Shoot B (ppm)
Caucasian clover		7.13	0.271	20.3	1.00	10.01
Lotus		11.25	0.240	28.8	0.40	14.42
Lucerne		9.36	0.228	22.7	0.58	12.30
Falcata lucerne		7.04	0.257	19.0	0.51	13.58
Strawberry clover		6.31	0.241	16.3	0.81	11.97
Tagasaste		10.68	0.217	24.3	0.68	6.33
White clover		6.14	0.288	18.8	0.82	12.98
Grand Mean		8.27	0.249	21.5	0.68	11.66
Species	SEM	0.170	0.0044	0.58	0.056	0.325
	LSD (5%)	0.475	0.0123	1.62	0.157	0.908
P Rate	0	5.39	0.181	9.5	0.69	10.36
	10	5.91	0.188	10.8	0.67	10.71
	30	6.72	0.200	13.2	0.66	9.80
	60	8.06	0.214	16.7	0.65	11.08
	100	8.93	0.229	20.0	0.73	10.46
	250	9.76	0.246	23.8	0.72	12.69
	500	10.70	0.293	31.1	0.64	12.59
	1500	10.70	0.439	46.5	0.71	15.56
	SEM	0.182	0.0047	0.58	0.060	0.348
	LSD (5%)	0.507	0.0132	1.73	0.168	0.971
P	Species	***	***	***	***	***
	P Rate	***	***	***	ns	***
	Sp*P Rate	***	***	***	ns	***

ns not significant, *** Significant at $P< 0.001$

Table 5. Rate of P application, equivalent Olsen P and shoot P concentration at which 97% of maximum yield was observed for each legume species.

Species:	P rate (mg P kg^{-1} soil)	Olsen P (mg L^{-1})	97% Maximum Yield (g DM pot^{-1})	Shoot P (%)
Arrow leaf clover	517	66	2.8	0.28 c
Balansa clover	888	108	2.2	0.36 b
Caucasian clover	439	57	9.7	0.32 c
Gland clover	1467	175	1.1	0.35 b
Lotus	440	57	14.3	0.31 c
Lucerne	669	83	11.7	0.29 c
Persian clover	369	48	12.9	0.25 d
Falcata lucerne	536	68	9.1	0.39 a
Sub. Clover	336	45	4.2	0.26 d
Strawberry clover	302	41	7.9	0.28 c
Tagasaste	132	21	12.6	0.23 d
White clover	202	29	7.8	0.32 c

Values with different letters are significantly different at $P< 0.05$.

3.1.4. Maximum yields (97%)

The 97% maximum DM yield of each species was calculated as a measure of biological maximum relative yield, and values are presented in Table 5. The maximum yield (97%) in combination with the P rate at this yield indicates P use efficiency. The Olsen P required for 97% of maximum DM yield differed between species but was high in all cases (Table 5). Gland and balansa clovers had very high optimum Olsen P values while Tagasaste and 'Nomad' white clover had the lowest values. Shoot P concentration differed between species ($P< 0.05$, Table 5). Optimum shoot P concentrations were highest for falcate Lucerne, balansa and gland clovers, and were lower for tagasaste, sub clover and Persian clover

3.2. Lime applications

3.2.1. Soil pH and exchangeable Al

The relationship between lime rate and pH, and soil pH and exchangeable soil Al are presented in Figures 3 and 4. The relationship between lime application rate and soil pH was best described by a quadratic function (Figure 3; $R^2 = 0.96$).
In general, for every 1.0 t ha^{-1} of lime applied soil pH increased by 0.16 units. Correspondingly, soil exchangeable Al concentrations decreased in a curvilinear fashion with increasing lime rate (Figure 4; $R^2 = 0.96$). Large increases in exchangeable Al were observed below soil pH 6.0.

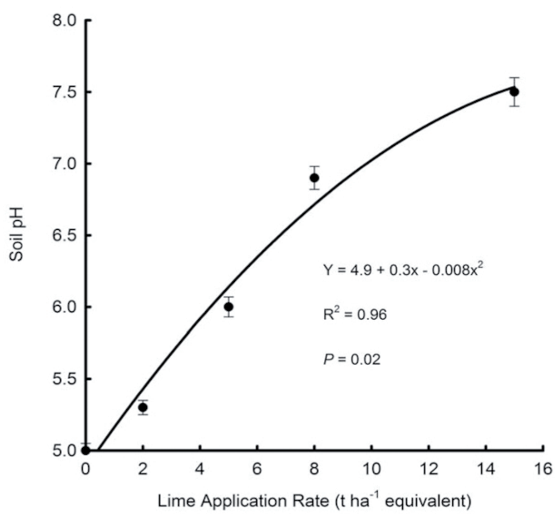


Figure 3. The relationship between lime application rate (5 levels of lime; ranging from 0 to 15 t lime ha^{-1} equivalent) and soil pH. Olsen P = 30 mg L^{-1}. Data are mean values ± SEM (n=20), with P and R^2 values for fitted curves showing data trend.

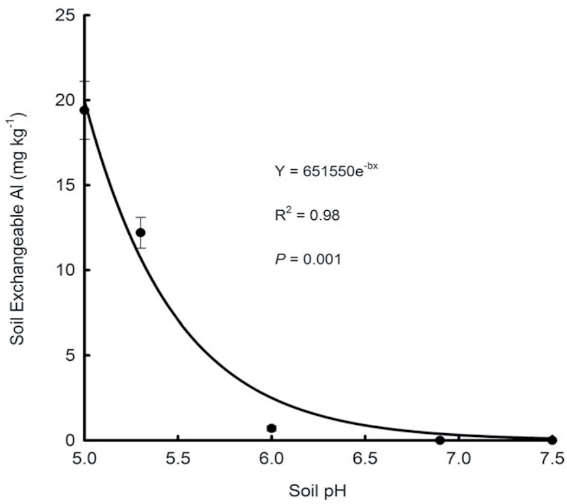


Figure 4 The relationship between soil pH and exchangeable soil Al. Olsen P = 30 mg L^{-1}. Data are mean values ± SEM (*n*=20), with P and R^2 values for fitted curves showing data trend.

3.2.2. Yield

A large difference (P< 0.001) in DM yield was observed as lime rate increased. The mean total shoot yield across all lime treatments ranged from 0.2 g DM pot $^{-1}$ (gland clover) to 11.0 g DM pot^{-1} (tagasaste) (Tables 5 and 6, Figure 2). Of the annual species, Persian clover was the highest yielding species .and lotus was the highest yielding perennial. In general the perennials yielded higher than the annual clovers due to their longer growth period. There was a strong (P< 0.001) species by lime rate interaction. This interaction effect indicates that the way in which the plants responded to the increased lime application rate differed among species. For this reason the relationship between increased lime rate and DM yield was examined in more detail using regression analysis of the data for individual species.

Again, the strong interaction effects are the key focus of the results and discussion of this experiment, rather than the main effects. Another main effect of lime was that the average concentration of phosphorus in the plant tissue increased (P< 0.001) up to a rate of at 5 t lime ha^{-1}, then decreased across all species with further lime (Tables 6 and 7).

As expected, mean Mo concentrations substantially increased across all species with increasing lime and boron concentrations decreased (P< 0.001).

All annual species increased in yield to a maximum point, at either 2 or 5 t lime ha^{-1}, then declined with further lime application (R^2 = 0.82 – 0.97) as soil acidity reduced (Figure 5). Arrow leaf clover stayed at near maximum (90%) yield across a greater range of lime rates

Table 6. Effects of lime rates on mean shoot yields, P concentrations, P uptakes, Mo concentrations, B concentrations and soil exchangeable Al concentrations for five annual pasture legume species.

Species:		Shoot Yield (g DM Pot^{-1})	Shoot P (% P)	Shoot P Uptake (mg P Pot^{-1})	Shoot Mo (ppm)	Shoot B (ppm)	Soil Exch Al (mg kg^{-1})
Arrow leaf clover		2.16	0.156	3.8	0.30	6.37	6.7
Balansa clover		0.64	0.183	1.3	0.60	10.68	6.5
Gland clover		0.24	0.107	4.6	0.23	6.77	6.3
Persian clover		6.12	0.191	12.1	0.92	14.77	5.8
Sub. Clover		1.54	0.193	3.2	0.54	9.30	6.9
Grand Mean		2.139	0.166	4.2	0.52	9.58	6.5
Species	SEM	0.103	0.0029	0.22	0.070	0.573	0.20
	LSD (5%)	0.289	0.0082	0.62	0.198	1.615	0.56
Lime Rate	0	1.13	0.177	1.9	0.13	16.47	19.4
	2	2.81	0.211	5.5	0.24	14.06	12.2
	5	3.12	0.208	6.7	0.58	7.69	0.70
	8	2.23	0.127	4.0	0.67	4.68	0.00
	15	1.41	0.107	2.7	0.96	4.98	0.00
	SEM	0.103	0.0029	0.22	0.070	0.573	0.20
	LSD (5%)	0.289	0.0082	0.62	0.198	1.615	0.56
P	Species	***	***	***	***	***	**
	L Rate	***	***	***	***	***	***
	Sp*L Rate	***	***	***	***	**	*

*Significant at *P*< 0.05, ** Significant at *P*< 0.01, *** Significant at *P*< 0.001

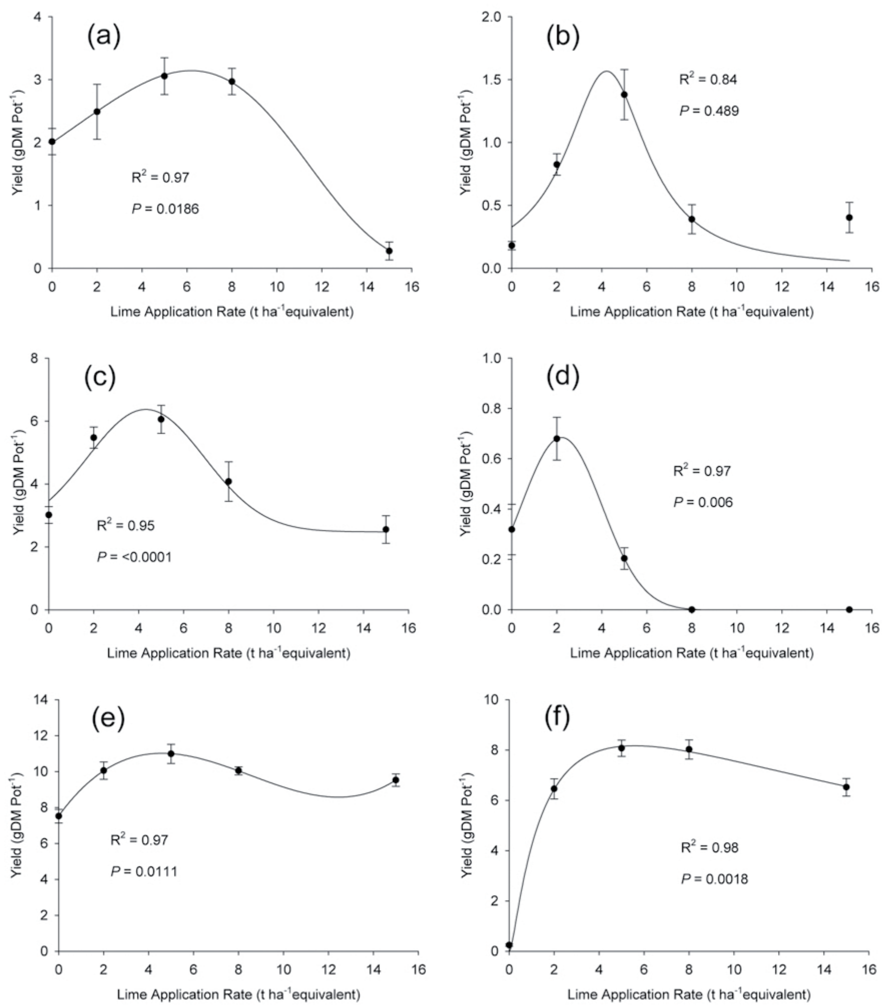
Table 7. Effects of lime rates on mean shoot yields, P concentrations, P uptakes, Mo concentrations, B concentrations and soil exchangeable Al concentrations for seven perennial pasture legume species.

Species:		Shoot Yield (g DM Pot^{-1})	Shoot P (% P)	Shoot P Uptake (mg P Pot^{-1})	Shoot Mo (ppm)	Shoot B (ppm)	Soil Exch Al (mg kg^{-1})
Caucasian clover		4.23	0.249	10.1	2.27	13.94	6.1
Lotus		9.62	0.233	22.5	0.49	17.09	6.0
Lucerne		5.86	0.194	12.1	0.71	16.31	6.4
Falcata lucerne		3.92	0.256	10.4	0.58	14.73	6.8
Strawberry clover		4.39	0.196	9.2	0.85	13.64	7.2
Tagasaste		7.05	0.203	13.2	0.59	8.32	5.8
White clover		3.94	0.243	9.6	1.27	16.14	6.3
Grand Mean		5.74	0.225	12.6	0.97	14.31	6.4
Species	SEM	0.165	0.0034	0.40	0.096	0.725	0.26
	LSD (5%)	0.461	0.0095	1.13	0.270	2.04	0.72
Lime Rate	0	2.90	0.189	5.5	0.08	23.96	18.5
	2	6.82	0.210	14.0	0.15	18.73	12.5
	5	7.57	0.238	17.7	0.72	11.94	0.9
	8	5.97	0.250	14.6	1.52	9.00	0.0
	15	4.62	0.236	10.9	2.37	6.70	0.0
	SEM	0.139	0.0029	0.34	0.081	0.613	0.22
	LSD (5%)	0.390	0.0081	0.96	0.228	1.72	0.60
P	Species	***	***	***	***	***	**
	L Rate	***	***	***	***	***	***
	Sp*L Rate	***	***	***	***	***	**

** Significant atP< 0.01, *** Significant atP< 0.001

(5.7 – 6.7) than the other annual species (Figure 5). The perennial species all increased sharply in yield with increasing lime inputs, then declined beyond around 4 t lime ha^{-1}. The exception was white clover which increased in yield sharply and then remained at that yield up to the maximum lime application rate (15 t lime ha^{-1}). Lotus, lucerne, strawberry clover and white clover remained near maximum yield across a wider range of lime rates then the other perennial species.

At 0 t lime ha^{-1} (pH = 5.0) tagasaste and lotus were the highest yielding species at 8.2 and 7.5 g DM pot^{-1}. Strawberry clover, balansa clover and lucerne were the lowest yielding at just 0.1, 0.2 and 0.2 g DM pot^{-1}. At 15 t lime ha^{-1} lotus, lucerne and Persian clover were the highest yielding species at 9.6, 6.5 and 5.5 g DM pot^{-1}. Gland clover, arrow leaf clover, balansa clover and subterranean clover were the lowest yielding species at 0.0, 0.3, 0.4 and 0.8 g DM pot^{-1}.



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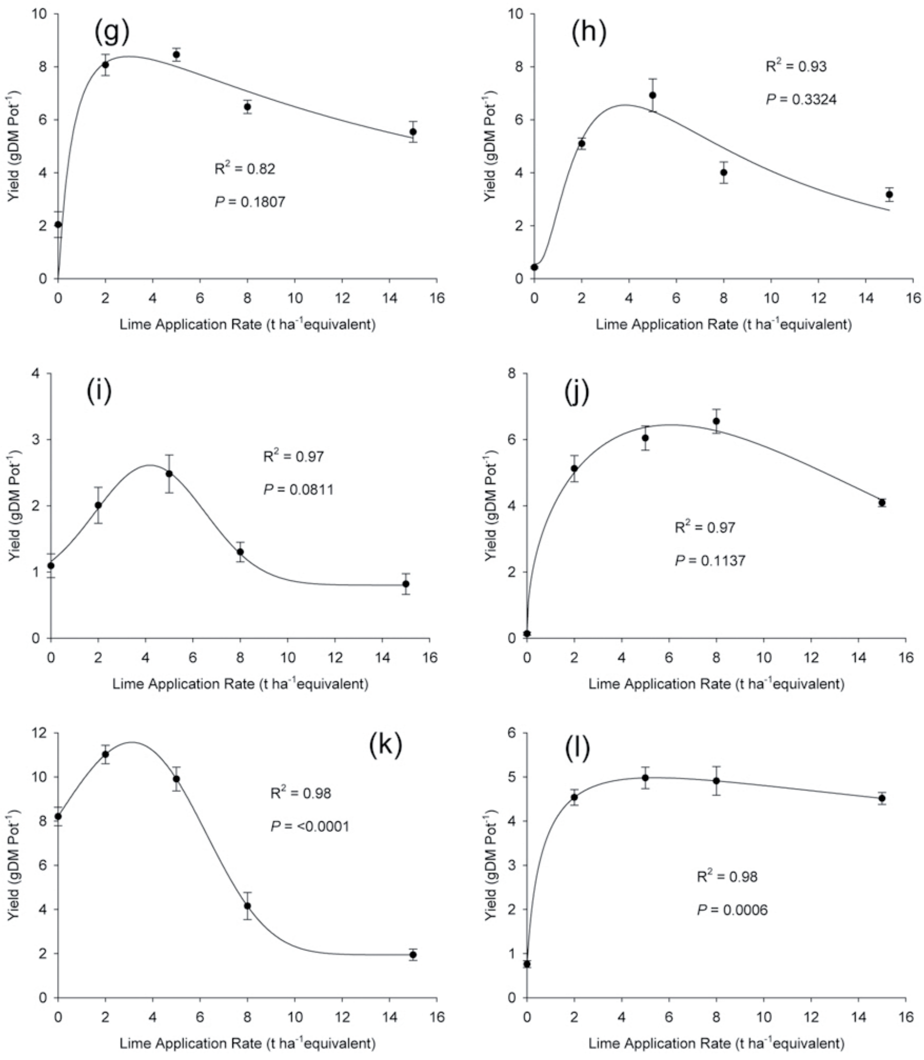

Figure 5. Total accumulated shoot dry matter (DM) yield response of pasture legume species (a) arrow leaf clover (*T. Vesiculosum*), (b) balansa clover (*T. michelianum*), (c) Caucasian clover (*T. ambiguum*), (d) gland clover (*T. glanduliferum*), (e) lotus (*L. pedunculatus*), (f) lucerne (*M. sativa*), (g) Persian clover (*T resupinatum*), (h) falcata lucerne (*M. falcata*), (i) subterranian clover (*T. subterranium*), (j) strawberry clover (*T. fragiferum*), (k) tagasaste (*C. proliferus*), (l) white clover (*T. Repens*) to increasing levels of soil lime (5 levels of lime; ranging from 0 to 15 t ha^{-1} equivilant), grown in an acid NZ high country soil. Data are mean values ± SEM (n=4), with P and R^2 values for fitted curves showing data trend (Line equations for all graphs are presented in the supplementary material).

3.2.3. Maximum yields (97%)

Optimum soil pH ranges varied between species, as did threshold soil exchangeable Al concentrations. (Table 8). Acid tolerant species included Persian and gland clovers and tagasaste, while Lucerne and arrow leaf and strawberry clovers were least tolerant. As expected, threshold soil Al concentrations were strongly associated with soil pH. Threshold soil Al values ranged from 2 (arrow leaf clover) to 8 (tagasaste) mg Al kg^{-1}. Of the annual species the greatest 97% maximum yield ranged from 1.1 g DM pot^{-1} (gland clover) at 2 t lime ha^{-1} to 8.1 g DM pot^{-1} (Persian clover) achieved at 2 t lime ha^{-1} (Table 8). Of the perennial species the 97% yield ranged from 4.8 g DM pot^{-1} at 3 t lime ha^{-1} (white clover) to 11.2 g DM pot^{-1} at 2 t lime ha^{-1} (tagasaste).

Table 8. Rate sof lime application at which maximum yield was observed for each legume species, including estimated pH range for 90% max yield and threshold soil exchangeable Al concentrations

Species	Lime Rate (t Lime / ha)	Optimum pH	97% Yield (g DM pot^{-1})	Threshold Soil Al Concentration† (mg kg^{-1})	Optimum pH range (+/- 10 % max yield)
Arrow leaf clover	5.0	6.0	3.1	2 b	5.7 - 6.7
Balansa clover	3.9	5.8	1.5	3 b	5.7 - 5.9
Caucasian clover	3.5	5.7	6.2	4 b	5.6 - 6.2
Gland clover	1.8	5.4	0.7	8 a	5.3 - 5.6
Lotus	3.0	5.6	10.7	7 a	5.4 - 6.5
Lucerne	3.8	5.8	7.9	3 b	5.6 - 7.0
Persian clover	2.0	5.4	8.1	8 a	5.3 - 6.3
Falcata lucerne	3.0	5.6	6.4	7 a	5.5 - 6.2
Sub. clover	3.5	5.7	2.5	4 b	5.6 - 6.0
Strawberry clover	4.3	5.8	6.2	3 b	5.6 - 6.8
Tagasaste	2.3	5.5	11.2	8 a	5.4 - 5.9
White clover	3.1	5.6	4.8	4 b	5.4 - 7.5

†Defined as; Soil exchangeable Al concentration at optimum pH. Values with different letters are significantly different at $P < 0.05$

4. Discussion

The objective of this study was to determine the yield and nutrient uptake response of twelve pasture legume species to P or lime additions when grown in an acid upland soil under glasshouse conditions. Strong interaction effects between legume species treatments were observed and as such are the focus of this discussion.

4.1. Phosphorus responses and critical P requirements

Increasing phosphorus inputs increased the yield of all annual and perennial legumes and strong differences among species were also observed. As expected, herbage P concentrations and total P uptake were also strongly influenced by P inputs. The annual species yielded less than the perennial species ($P< 0.001$) with the exception of Persian clover due to their shorter growth period, as annual species go to seed, die and re-establish from seed each year Olsen P increased with increasing P inputs. It required 7.9 kg P ha^{-1} to increase the Olsen P by one unit when the soil had been limed to a pH of 6.0. Morton and Roberts (1999) state that to raise the Olsen P by one unit, you require between 4.0 and 7.0 kg P ha^{-1} on a sedimentary soil. Our result was just above this range, which is typical of capital fertilizer inputs required when shifting P levels from low to high status. The soil Olsen P at which maximum yield was achieved differed substantially between legume species, but values were high in all cases. Species differences were likely driven by genetic adaptations to low phosphorus environments (Maxwell *et al*., 2013) and therefore each have different P requirements and P use efficiencies for growth (Raven *et al*. 1992, Syers *et al*. 2008). Tagasaste and 'Nomad' white clover peaked at the lowest Olsen P's, while Lucerne and balansa and white clover required very high Olsen P for peak yield.

Critical shoot P concentration (shoot % P at peak yield) also differed significantly between species, ranging from 0.23% in tagasaste to 0.39% in falcate lucerne. It is also important to take account of the maximum yield of the legumes when interpreting these results.

Pang *et al*. (2009) reported that lucerne and lotus had relatively low concentrations of shoot P with increasing P supply (maximum of 10 and 12 ppm P respectively). This is similar to the results of this experiment, and confirms their suitability to low phosphorus environments, indicating that the tissue has a low demand for phosphorus compared with some other species. Dodd and Orr (1995b) found that white clover on average had a P concentration of 0.21%, which is comparable with the low P treatments of this study. Of the annual species subterranean clover and Persian clover reached their biological optimum yields at much lower P rates than gland and balansa clovers. Of the perennial species, tagasaste, white clover and strawberry clover achieved biological optimum yields at much lower P rates than for lucerne and falcata lucerne. Beyond these P rates, yield did not increase, suggesting that factors other than soil P availability began to limit yield. Of the annual species the Persian clover was the highest yielding with no P input, which was higher than the other annual species under these conditions. Lotus, tagasaste and lucerne were the highest yielding of the perennial species with no phosphorus inputs.

This result indicates that Persian clover, lotus, tagasaste and lucerne are able to be very productive, even in low P fertility upland soils, when compared to the other species in this experiment. Although a growth response to P was expected, this has not been documented in scientific literature for many of the species examined in this experiment. As such this data represents new and valuable information for most of the legume species examined in this experiment.

Caradus (1980) conducted a similar experiment, investigating the effect of P on 'Treeline' Caucasian clover, 'Palestine' strawberry clover, 'Maku' lotus, 'Woogenallup' subterranean clover and 'Huia' white clover. Comparable phosphorus rates were used (300 and 2000 mg P kg soil^{-1}) in pots of naturally P deficient soil over 24 weeks, however only six weeks of growth data were presented. Their yields were significantly less than those found in this experiment. Subterranean clover out-yielded lotus in their experiment which contrasts our result, but this was most likely due to the short duration of their experiment. The soil used in that experiment also had a very high phosphorus retention capacity. Caradus (1980) also found that lotus yield was less affected by P inputs in terms of an increase in yield, with a 36% increase in yield, compared with a 46% increase in this experiment. They found that there were no significant increases in yield between 400 and 500 mg P kg soil^{-1}, while the yield increased from 0 to 400 mg P kg soil^{-1} for 119 different cultivars of white clover. This contrasts the results of this experiment where 'nomad' white clover continued to increase in yield up to 1500 mg P kg soil^{-1} in our experiment. Hart and Jessop (1984) found that 'Maku' lotus out yielded white clover across all P application rates up to 2000 mg P kg soil^{-1} which agrees with our findings. This is likely due to lotus having superior root growth compared with white clover and therefore greater opportunity to infiltrate the soil volume and utilise soil P (Scott and Lowther 1980). Dodd and Orr (1995a) found similar results, looking at the phosphorus response from 18 annual legume species at low soil pH (5.4) with two rates of P fertility (Olsen P = 10 and 24 mg L^{-1}). They found that subterranean clover, balansa clover and arrow leaf clover all yielded more with improved phosphorus status in the soil. The P requirements for optimum growth of these pasture legume species were often at very high Olsen P values, well beyond 'typical' farmer fertiliser inputs under field conditions.

4.2. Lime response and soil Al thresholds

Lime additions also had a strong effect on the DM yields for all the legumes in this experiment, and importantly, affected species differently This was driven by a reduction in soil exchangeable Al concentrations with increasing lime which reduced phytotoxicity. With the control soil at a pH of 5.0, lime addition to the soil was expected to reduce the exchangeable aluminium levels in the soil, while increasing the P and Mo availability to the plants up to a pH of 6.0 (Wheeler and O'Conner, 1998).

Lime inputs increased the soil pH by 0.16 pH units per t of lime ha^{-1} applied suggesting a low pH buffering capacity, most likely due to the low organic matter content of this soil. Morton and Roberts (1999) state that 1.0 t of quality lime ha^{-1} will raise the pH by 0.1 unit, on ash and pumice soils. This soil required much less than these recommendations, although 100% $CaCO_3$ was used in this experiment. Agricultural lime is more likely to be 80% $CaCO_3$.

Optimum pH is determined by the point at which no further response to lime occurs (Edmeades *et al.* 1984) for plant production. Optimum soil pH differed between species, which was strongly associated with soil exchangeable Al concentrations. Tagasaste demonstrated a propensity for acid soils and appeared unaffected by soil Al concentrations of 8 mg kg^{-1}. In contrast, lucerne, balansa and strawberry clovers were acid / Al sensitive. The yields of these latter species declined when exchangeable Al increased above 3 mg kg^{-1}. Although the impact of Al on this suite of legume species has been examined previously (e.g. Edmeades *et al*. 1991) such studies have been limited because the plants have not been grown in soil.

As such, this experiment provides the first and vital new information on the Al sensitivity of this suite of legumes when grown in a soil environment, under a range of soil pH / exchangeable Al regimes. The next critical stage of this research is to confirm the results of this glasshouse study under field conditions.

In addition, strawberry clover, lucerne and falcata lucerne had large increases in yield from the control compared to other species such as tagasaste and lotus which only had moderate increases in yield under optimum liming treatments. This may have been driven by adaptations of some species to these acidic environments, potentially making these species more suitable for acidic upland farms than others (Maxwell *et al*. 2014). Species exhibiting very large lime responses are those which are particularly sensitive to low soil pH, in terms of Al toxicity, and P and Mo deficiency. Pasture legumes are considered deficient in molybdenum at levels below 0.10 mg kg^{-1} (Morton *et al*. 1999, Mengel and Kirkby 2001). Although a growth response to lime was expected in some species (e.g. lucerne, Scott *et al*. 2008; sub clover, Bouma *et al*. 1981), this has again is undocumented in scientific literature for several of the species examined in this experiment. Therefore much of this represents new information for these species.

Corero and Blair (1978) reported that subterranean clover had no increase in yield between a pH of 4.4 and 5.9 in a super phosphate trial on a coarse textured gravely sand soil in a glasshouse trial. That conflicts with the findings of this study, most likely due to the differences in soil used. Edmeades *et al*. (1991) found that while subterranean clover was not sensitive to low pH, it was moderately sensitive to Al toxicity. This soil at the control pH of 5.0 had a high exchangeable Al concentration and therefore the exchangeable Al was reduced with increasing pH, and thus increased yield. In a field trial, Lambert and Grant (1980) found that under large (3.5 t lime ha^{-1}) liming treatments in North Island hill country, pasture legume yield decreased from the control. This was attributed to a depression in the P availability in the soil at a high pH (> 6.0) due to calcium phosphate formation (Larsen *et al*. 1965).

5. Conclusions

Phosphorus inputs significantly increased the yields of all annual and perennial species. The highest yielding annual species was Persian clover at both low and high P inputs and the highest yielding perennial species was lotus at both low and high phosphorus rates. while white and strawberry clovers were the lowest yielding perennial species. Yield increases were driven by increasing soil P availability and therefore increased plant P uptake. The P rate at which maximum yield occurred varied greatly between species, suggesting large differences in P use efficiency between species due to adaption to low soil P environments. Optimum shoot P concentrations were identified for all species.

Lime significantly increased the yields of all annual and perennial species up to a maximum, where yields then decreased with further lime additions. The optimum pH ranges for plant growth were identified, which varied between species. The highest yielding annual species was Persian clover while the lowest yielding annual species was gland clover under optimum pH conditions. The highest yielding perennial species was tagasaste (pH 5.3) and lowest white clover (pH 5.6) at optimum pH. Yields were driven primarily soil exchangeable Al concentrations, which in turn were driven by soil pH. Trace elements also had some effect on yield, as with increasing pH, the amount of plant available Mo increased, while plant available B decreased. There were significant differences between species in terms of lime effects, which

indicated that these species differ in their optimal pH growth conditions and also the range of pH conditions in which they perform best.
This experiment has identified the optimum soil phosphorus and threshold soil exchangeable Al concentrations for these species, of which for many this is new and valuable information. The suitability of these species must now be researched under field climatic and physical conditions.

Acknowledgements

The authors wish to thank; Agam Nangul, Malcolm Smith and the Glasshouse staff for technical assistance, Angela Reid and Julia Bellamy for assistance with herbage digests and Lynne Clucas for ICP analysis.This project was funded by the Lincoln University Research Fund (LURF).

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